ON THE EFFECT OF VARIATIONS IN THE TEMPERATURE UPON THE PROCESS OF ARTIFICIAL PARTHENOGENESIS.

ARTHUR W. GREELEY.

In a previous paper[1] it has been shown that the unfertilized eggs of *Asterias* may be made to develop parthenogenetically by exposing them for a definite length of time to a temperature of 1° C. to 7°C. These experiments were the outcome of others[2] in which it had been demonstrated that the cells of *Spirogyra* and some Protozoa may be made to lose water by lowering the temperature. From these observations on the effect of low temperature on simple animal and plant cells, it is made probable that the artificial development of the *Asterias* egg by means of a lowering of the temperature is brought about by an extraction of water from the protoplasm, just as if the eggs had been placed in a solution of a higher osmotic pressure than that of the sea-water. This latter method has been found to be successful for the *Asterias* as well as the *Arbacia* egg by Dr. C. H. Neilson. Beyond the fact that a low temperature brings about certain changes in the physical condition of the protoplasm that result in a loss of water, nothing is known concerning the action of low temperatures upon the egg.

In the course of experiments on artificial parthenogenesis it has been noted by several observers that the experiment must be performed within certain very narrow limits of temperature. Above or below these limits of temperature, of which 20° C. may be taken as the mean, the experiments have failed with any of the methods heretofore used to obtain artificial parthenogenesis. In order to determine, if possible, the rôle which temperature plays in this process, Dr. Loeb suggested that I repeat some of the well-known experiments in artificial parthenogenesis, test-

[1] Greeley, *American Journal of Physiology*, VI., 1902, p. 296.
[2] Greeley, *ibid.*, VI., 1901, p. 122.

ing the effect of the solution upon the egg at different temperatures.

All the salt solutions used in the experiments on artificial parthenogenesis may be divided into two classes according to their supposed action upon the protoplasm: first, those solutions used at a higher concentration than that of sea-water which produce parthenogenesis through the extraction of water from the protoplasm; and, second, those solutions used at the same or at a less concentration than that of the sea-water which affect the egg through the specific action of ions. In the light of the observations already made on the effect of changes in temperature upon protoplasm, it was a point of considerable interest to determine whether variations in the temperature would affect the action of each of these classes of solutions upon the egg in the same way. Since the speed of a chemical combination varies directly with the temperature, it might be supposed that the specific action of an ion upon the egg would be inhibited or slowed by lowering the temperature, and accelerated by raising the temperature, up to the point where the protoplasm begins to go into heat-rigor. And similarly, since a reduction of the temperature extracts water from the protoplasm, the action of concentrated solutions upon the egg ought to be increased by a lowering of the temperature. Such a disparity between the effect of variations in the temperature upon the chemical and osmotic methods of producing artificial parthenogenesis was, however, not found to hold. A lowering of the temperature, for example, inhibits or slows the action of all solutions upon the egg, whether they are isotonic or hypertonic to sea-water.

The experiments were performed at Woods Hole, during the summer of 1902, with such temperatures as could readily be obtained in the laboratory with the aid of an ordinary hot-air oven and a refrigerator. Four temperatures were used; namely, 30° C., 23° C., 11° C. and 2° C. The solutions to be used in the experiments were prepared and divided among four dishes. The dishes were placed at these four temperatures, and allowed to stand forty-five minutes before the addition of the eggs. The eggs were then distributed among the various dishes at the different temperatures, and after certain intervals of time, were re-

moved to fresh sea-water at the temperature of the room. After removal to the room temperature the eggs were carefully examined from time to time to determine the exact proportion of segmentations and swimming larvæ produced by the same solution at the four temperatures mentioned above. Great care was taken that, with the exception of temperature, all the conditions affecting each lot of eggs might be identical.

The following eggs and methods were used: *Arbacia* eggs, action of concentrated solutions of $MgCl_2$, NaCl, and of sea-water concentrated by evaporation; *Asterias* eggs, action of acids and a concentrated solution of KCl; *Amphitrite* eggs, action of $Ca(NO_3)_2$. Each solution was tried at the four temperatures mentioned above. All these solutions, with the exception of the acids and the $Ca(NO_3)_2$, are of a higher osmotic pressure than the sea-water, and affect the eggs through the extraction of water. In the case of the acids and $Ca(NO_3)_2$, the osmotic pressure remains unchanged, and the fertilization is ascribed to the specific action of the H or Ca ion.

We will consider first the effect of variations of temperature on the process of artificial parthenogenesis when produced by those solutions which extract water from the egg.

Experiment 1.—*Arbacia* eggs were placed in the following solution: 50 c.c. $2\frac{1}{2}n$ $MgCl_2$ plus 50 c.c. sea-water, and were removed to normal sea-water at intervals of 1, 2, $3\frac{1}{2}$ and 5 hours. The following temperatures were used: 30°, 23°, 11°, 2° C. Only a small proportion of swimming blastulæ were formed at a temperature of 23° C. and about half as many at 11° C. No development took place at 30° C. or 2° C. This was not a successful experiment, as only a small percentage of eggs segmented at all. An increase and a decrease in temperature, to 30° C. and 2° C. served equally well to entirely inhibit segmentation.

Experiment 2.—*Arbacia* eggs were placed in the following solution: 11 c.c. $2\frac{1}{2}n$ NaCl plus 89 c.c. sea-water. Same temperatures and periods of exposure to the solution as in experiment 1. The results of this experiment will be given in tabulated form as follows:

	Periods of Exposure to Solution.			
	1 hour.	2 hours.	3½ hours.	5 hours.
30°	0	0	0	0
23°	5	10	0	0
11°	0	5	15	20
2°	0	·5	·5	10

The figures indicate the number of swimming blastulæ formed in 100 eggs. At a temperature of 30° C. no development took place as a result of the NaCl solution, except a few irregular segmentations. At 11° C. a slightly longer residence in the solution over that necessary at the room temperature (23° C.) was required, although at periods of three to five hours a larger percentage of blastulæ were formed than at the room temperature. At a temperature of 2° C. a still longer exposure to the solution was required to produce any effect. At this temperature practically no development took place until the eggs were exposed to the solution for five hours. At 30° C. only a few irregular segmentations occurred as a result of the NaCl solution.

Experiment 3.—*Arbacia* eggs were placed in the following solution: 100 c.c. sea-water concentrated by evaporation to three fourths its volume. This solution has the same relative proportion of ions as normal sea-water, and hence its only effect upon the egg can be the purely physical one of extracting water from it. The same temperature and periods of exposure were used as in experiments 1 and 2. The results will be presented in tabulated form as in experiment 2.

	Periods of Exposure to Solution.			
	1 hour.	2 hours.	3½ hours.	5 hours.
30°	0	0	0	0
23°	0	4	·5	0
11°	0	15	25	20
2°	0	·5	·4	3

Many more of these eggs segmented at the room temperature (23° C.) than is indicated in the table, but the cells fell apart before complete blastulæ were formed. Practically the same general result is seen as in experiment 2. The optimum period of exposure to the concentrated sea-water becomes increasingly longer as you lower the temperature, but with these longer

periods of exposure and low temperatures, a larger percentage of blastulæ are formed than at the temperature of the room. An increase in temperature to 30° C., as in experiment 2, entirely inhibited the development of the egg.

Experiment 4.—*Asterias* eggs were placed in the following solution: 15 c.c. 2½*n* KCl plus 85 c.c. sea-water. This method was suggested to me by Dr. C. H. Neilson. Same temperatures were used as in the previous experiments. The eggs were removed from the solution after the following periods: 5 min., 15 min., 30 min., 45 min., 1 hour and 2 hours. The results are tabulated as follows:

PERIODS OF EXPOSURE TO SOLUTION.

	5 min.	15 min.	30 min.	45 min.	1 hour.	2 hours.
30°	1	1	0	0	0	0
23°	20	30	5	0	0	0
11°	0	5	25	20	3	1
2°	0	0	0	.2	1	10

This experiment shows very clearly the inhibiting action of low temperatures on the process of artificial parthenogenesis at short periods of exposure to the solution. The period of exposure necessary to produce development increases steadily as the temperature is lowered, although the optimum period of exposure at any temperature varies greatly according to the maturity of the eggs. The development produced at a temperature of 2° C. after two hours' exposure, was shown not to be due to the low temperature alone by control experiments in which eggs in normal sea-water were kept at this temperature. No development by means of low temperature alone can be obtained, unless the eggs are exposed to the low temperature from three to five hours. At 30° C. again practically no development occurred.

We now turn to the effect of variations in the temperature on the process of artificial parthenogenesis when produced by the action of specific ions.

Experiment 5.—*Asterias* eggs were placed in the following solution: 5 c.c. *n*/10 HCl plus 100 c.c. sea-water. This is a method[1] which has been elaborated by Loeb and Neilson and with favorable lots of eggs yields a very high percentage of de-

[1] Loeb, Fischer and Neilson, *Archiv für die ges. Psychologie*, 1901.

velopment. The same temperatures and periods of exposure were used as in experiment 4. The results are tabulated as follows:

	Periods of Exposure to Solution.					
	5 min.	15 min.	30 min.	45 min.	1 hour.	2 hours.
30°	2	0	0	0	0	0
23°	30	20	1	0	0	0
11°	0	75	75	50	25	0
2°	0	4	15	20	30	2

Practically the same result was obtained as with the KCl solution. The low temperature slows the action of each solution about equally, although the KCl solution extracted water from the egg and the acid solution affects the egg only through the specific action of the H ion. As in the previous experiments, an even larger percentage of larvæ was produced with a low temperature and long exposure, than at the temperature of the room. A temperature of 30° C. had the same inhibiting effect as in the preceding experiments.

Several experiments were performed on the effects of different temperatures upon the process of artificial parthenogenesis in the *Amphitrite* egg when produced by the specific action of the Ca ion. The following solution was used: 4 c.c. *n* $Ca(NO_3)_2$ plus 96 c.c. sea-water. Results were obtained in general similar to those already described for *Arbacia* and *Asterias*, although they were not nearly so uniform. Artificial parthenogenesis occurs at all the four temperatures tried with periods of exposure as short as fifteen minutes, but the optimum period of exposure is much longer at the low temperatures than at the temperature of the room.

In a previous paper[1] I described some experiments on the effects of an increase in temperature alone upon the unfertilized *Asterias* eggs. In no case did I get even a segmentation of the egg by an increase in temperature. These experiments were repeated this summer upon the eggs of *Amphitrite* and *Asterias* with the same result. I modified the experiment in many ways, keeping some of the eggs constantly at a slightly higher temperature than that of the room, while others were returned to normal sea-water after varying periods of exposure to temperatures ranging from 27° C. to 35° C. The protoplasm of the *Asterias* egg

[1] Greeley, *American Journal of Physiology*, VI., 1902, p. 296.

exists in a very delicate condition of equilibrium as regards its relation to the surrounding temperature. An increase in temperature of only 3° C. over that of the room (24° C. to 27° C.) suffices to liquefy the protoplasm of the egg. Within two hours after the eggs have been exposed to this increase in temperature, the protoplasm loses its granular appearance, becomes clear and homogeneous and flows out, greatly extending the egg membrane. At the same time or before the process of liquefaction has become complete, the nuclear wall breaks down or goes into solution, and the eggs in this stage appear to contain from two to thirty or more nuclear fragments scattered throughout the cell. After six to eight hours' exposure to this increase in temperature, the protoplasm goes into heat-rigor, but beyond the fragmentation of the nucleus, in no case was there even a semblance of segmentation.

This exceedingly delicate condition of equilibrium as regards the physical condition of the protoplasm of the unfertilized *Asterias* egg and its relation to the surrounding temperature, makes it so sensitive to any increase in the temperature, that is seems well-nigh impossible to cause the segmentation of the egg by that means.[1] The subject is by no means closed, however, and further experiments will be performed along this line.

This profound change in the physical condition of the protoplasm as a result of a very slight increase of temperature may explain the fact noted in all the descriptions of the experiments in this paper, that at a temperature of 30° C., artificial parthenogenesis cannot be produced with any of the methods heretofore used. Even at a temperature of 27° C. the protoplasm of the *Asterias* egg becomes completely liquefied, and in this condition no segmentation of the egg can occur.

Summary.

1. The length of exposure to a solution necessary to produce artificial parthenogenesis of the unfertilized eggs of *Asterias* and *Arbacia* varies inversely with the temperature. This applies to

[1] In a recent paper, however, Delage (*Archives de Zoöl. Exper.*, 1902) describes experiments in which he obtained artificial parthenogenesis of the *Asterias* egg by raising the temperature.

all the solutions used, whether they exert a chemical or an osmotic effect upon the egg. But, at the same time, with lower temperatures and longer periods of exposure to the solution, a larger percentage of larvæ are formed than at the temperature of the room.

2. An increase of temperature to 27° C. liquefies the protoplasm of the *Asterias* egg, and produces a fragmentation of the nucleus. At 30° C. it was found impossible to produce artificial parthenogenesis in *Asterias* or *Arbacia* with any of the solutions used.